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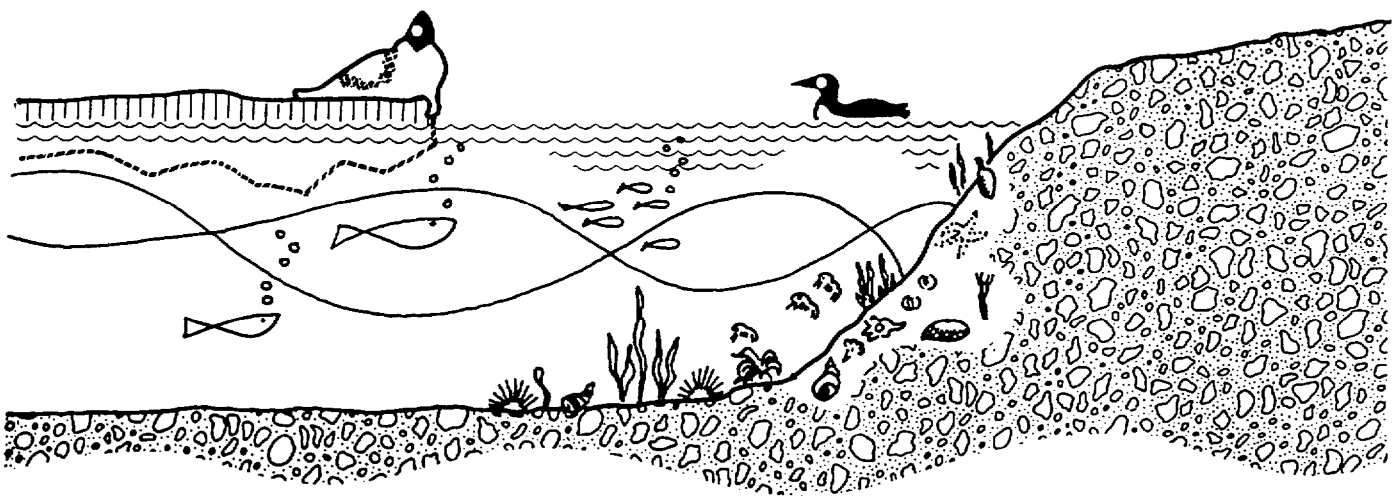
MICROBIOLOGY

2. Biodegradation of Oil



Baffin Island Oil Spill Project

WORKING REPORT SERIES

1980 STUDY RESULTS

BIOS Working Report Series

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BAFFIN ISLAND OIL SPILL PROJECT

MICROBIAL DEGRADATION OF OIL

MEASUREMENTS IN RAGGED CHANNEL, Z-LAGOON AND ECLIPSE SOUND, CAPE HATT 1980, A BASELINE ASSESSMENT,

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ABSTRACT

During the period 26 August to 9 September 1980 analyses of baseline parameters related to microbial degradation of oil were determined in samples from the nearshore waters and sediments of Ragged Channel (Bay 9, 10 and 11) and in shoreline sand and sediments inside and outside the Z-lagoon of Cape Hatt.

Most probable number techniques were used to assess counts of oildegrading microorganisms (bacteria), generally heterotrophic bacteria and antibiotic resistant oildegrading microorganisms (fungi). An experimental laboratory technique was used to determine rates of mineralization of generally tritiated Lago Medio crude oil and ^{14}C-labelled *n*-hexadecane, naphtalene and benz(a)pyrene. As a measure of general biological activity in shoreline sand and sediments a field method for determining CO_2-production.

In the coastal water an average content of $4 \cdot 10^4 L^{-1}$ oildegrading bacteria was detected, constituting about 1% of the assessed viable count for generally heterotrophic bacteria. Only 5 bottom sediments were analyzed. They contained oildegrading bacteria in the range 2,5”103 to 2,5.104 ml^{-1} sediment.

Experiments with tritiated Lago Medio demonstrated mineralization of oil by the microorganisms in the water and maximal rates (V_{max}) from 11 to 30 $\mu g/m^3$ water, d. were measured. Tentative results indicated a somewhat lower activity for mineralization of *n*-hexadecane. Definite mineralization of naphtalene and benz(a)pyrene needs confirmation.

The shoreline sand and sediment contained low but detectable numbers of oil degrading microorganisms. After spraying with weathered Lago Medio crude oil or the same oil as 50% water-oil emulsion an increase in oil-degrading bacteria from-102 to 105 respectively 107 cells per ml sand/oil mixture could be registered over a period of 15 days.

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1. INTRODUCTION

The participation of the Norwegian microbiology group in the BIOS-project has two major general objectives which can be phrased through these questions:

1. How will the microbial community of the cold waters of the Arctic coast react to a massive exposure to oil? How fast and to what extent will the microbial potential for oil degradation change? And, in this context, will the physical state of the oil as slick or as a dispersion have any significant influence on the microbial response?

2. Under the harsh condition of the Arctic shoreline is it still possible within the limits of practicality to enhance the normal potential of the shoreline for microbial degradation of stranded oil? We are thinking along two lines of approach: a) increased levels of nutrients, b) improved conditions for aeration.

Our work in 1980 was aimed at

- testing our methods of analysis and experimentation in the field and to gain experiences for work in the Arctic.

- to assess the baseline levels of oildegrading microorganisms and generally heterotrophic microorganisms in the nearshore waters and sediments of Ragged Channel and in the sand and tidal sediments of the shorelines inside and outside the Z-lagoon.

- to assess the biochemical activity for mineralization of radiolabelled oil and hydrocarbon in water and bottom sediments.

- to start and monitor the initial phase of the first experiment related to the fate of oil stranded and washed up on the shoreline.

This report summarizes our activities and results from the fieldwork at Cape Hatt 23 August to 15 September 1980.

2, MATERIALS AND METHODS

2.1.1. Water samples.

Water samples were taken from Zodiac by using a 5 l Niskin bottle. The bottle was rinsed by surface water at each station prior to the first sample. The sampled water was immediately dispersed into sterile flasks or sterile plastic bags (for large water samples), brought to the lab and stored at $+2^{o}C$.

At each station samples were normally taken at lm, 5m and 10m depth. In Bay 9, 10 and 11 stations H1 to H6 (see map on Fig. 1) were sampled according to the schedule given below and the main sampling period coincided with the cycle 3 of Canadian microbiology and the analysis program for environmental chemistry. Station number, date and depth specifically identify the samples concomittently analyzed by all three partners.

For comparative purposes on one occasion water samples were taken at the west shore of the Ragged Channel; these stations are designated S1 - S3 (see Fig. 1).

Water sampling period:

Date:	8/26	8/28	8/30	9/1	9/3	9/4	9/9
Station:	H1	Hi-2	H3-4	H5-6	H1	H4	S1-3

|—— Cycle 3 ——|

2.1.2. Sediment and beach sand samples.

One sample from subtidal Channel area (H2 - 8/29) was taken with a Peterson gravity sampler from Zodiac. The other sediment samples analyzed by us were recovered by divers from 7 m depth, by scooping sediments from the top 2 cm layer at convenient locations into a plastic container. The samples were stored at $+2^{O}C$ and analyzed within 2-3 hours. In one case (H1 - 9/2) the sample was frozen and thawed the next day immediately prior to the analysis.

For sampling sand from beaches (oiled or not oiled) and sediment from tidal areas the tip of a sterile disposable syringe was cut off leaving a syringe barrel with full opening. By inserting the syringe into the sand a measured volume of sand, usually 5 ml, could be collected. In some cases several 5 ml samples from the same general area were mixed in a sterile plastic beaker, and a 5 ml collective subsample was taken in the same manner described above. In most cases a collective 5 ml sample was prepared directly by filling the initial syringe with sand from 4-6 sampling locations. Both

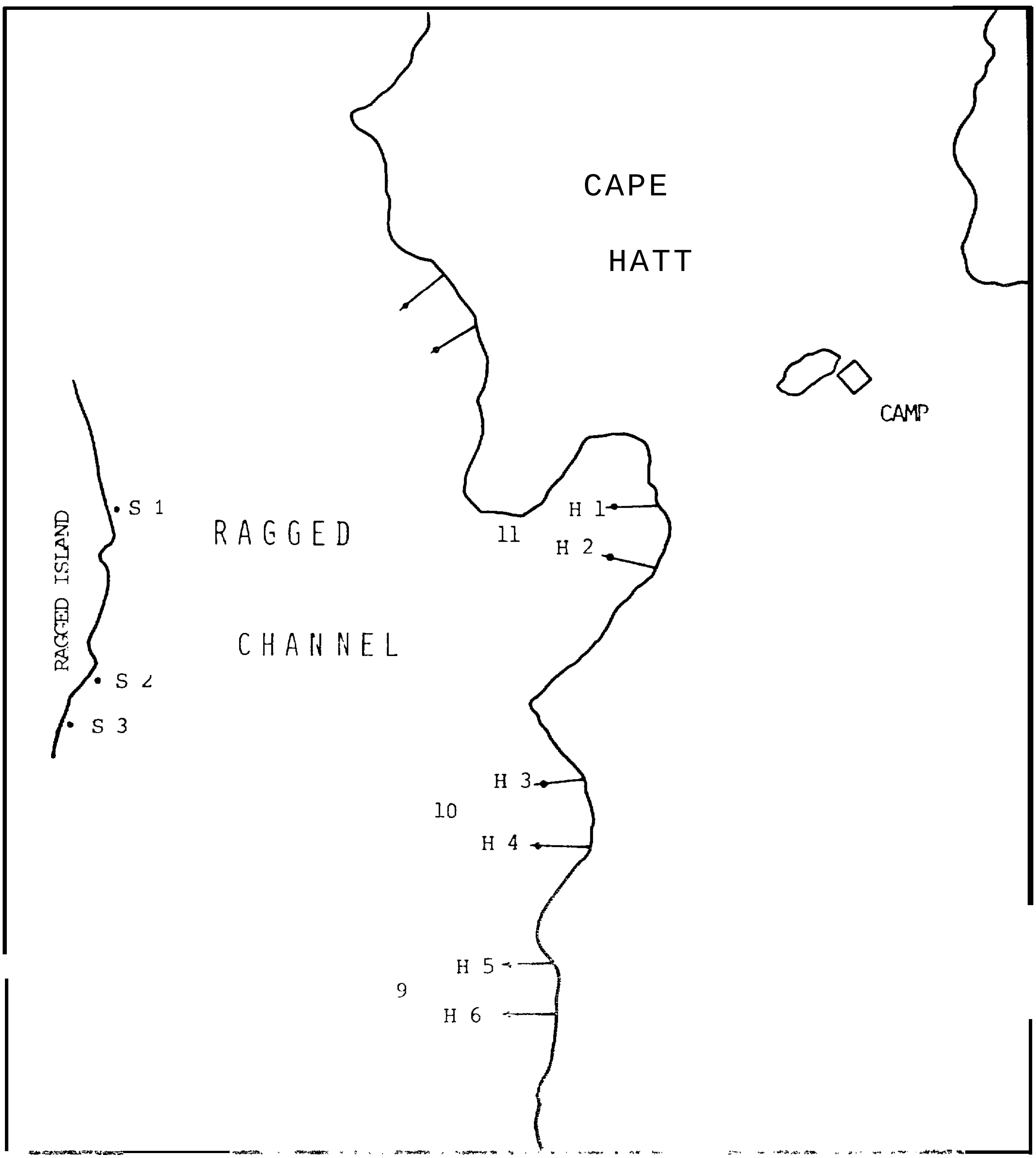


Fig. 1. Location of sampling stations H 1 - H 6 and S 1 - S 3 in Ragged Channel, Baf f in Island; August/September 1980.

types of collective samples were brought to field lab in a sterile 50 ml plastic container for analysis.

2.2. EXPERIMENTAL AND ANALYTICAL METHODS

2.2.1. Radioactive chemicals.

Generally tritiated Lago Medio crude oil (3H-Lago Medio) was prepared by The Radiochemical Centre, Amersham, England, by exchange reaction with 3H20. Components of the crude oil with boiling points below 200^{o}C had been removed prior to the tritiation and the final product corresponded to a medium weathered oil and had a specific activity of approx. 1 m C_i/mg. Applied to a column of activated silica the 3H-Lago Medio could be separated into three fractions in the usual way by successive elutions by heptane, benzene and methanol. Fig. 2 gives the graphic profile for the distribution of radioactivity in the various fractions eluted from the column. The specific activity by weight in the alkane fraction (eluted by heptane) was substantial, but as expected somewhat (about 40%) lower than in the more polar aromatic fractions.

1-^{14}C- *n*-hexadecane (sp.activ 235 µC_i/mg), [1(4,5,8)-^{14}C] - naphthalene (sp.activ. 40 µC_i/mg) and [7,10-14CI - benz(a)pyrene (sp.act. 86 µC_i/mg) were purchased from The Radiochemical Centre, Amersham, England. Solutions of appropriate concentrations and specific activity were prepared by dissolving "hot" and when required cold chemical in cyclohexane (^{3}H-Lago Medio), hexane (^{14}C- *n*-hexadecane), and toluene (^{14}C-naphthalene and benz(a)pyrene). All four radioactive substrates were at times used at two levels of specific activity, 100% and 1%. Except for the *n*-hexadecane which was diluted to 100 µC_i/mg the 100% corresponded to the specific activity of the commercial products.

2.2.2. Hydrocarbon mineralization experiments.

2.2.2.1. Preparations of water and sediment samples.

The water for assessment of the biochemical activity for mineralization of hydrocarbons was taken from 5 m depth at all stations. 500 ml (in some cases 1500 ml) of water was filtered through sterile 50 mm polycarbonate membrane filters (Uni-Pore, pore size 0,4 µm), the filter roiled up and immediately deposited into a 120 mm sterile screwcapped tube containing 10 ml of the same sample water. The screwcap had an inner liner of aluminum. As the filter folded out and lined the wall of the tube, the surface containing the particles including the bacteria retained from the filtered water sample faced the inside of the tube.

For analyses of sediments the following technique was used. 5 to 20 ml sediment sample was suspended to 50 ml with freshly sampled water from the 10 m depth of the same station and 1 ml samples of the resulting slurry

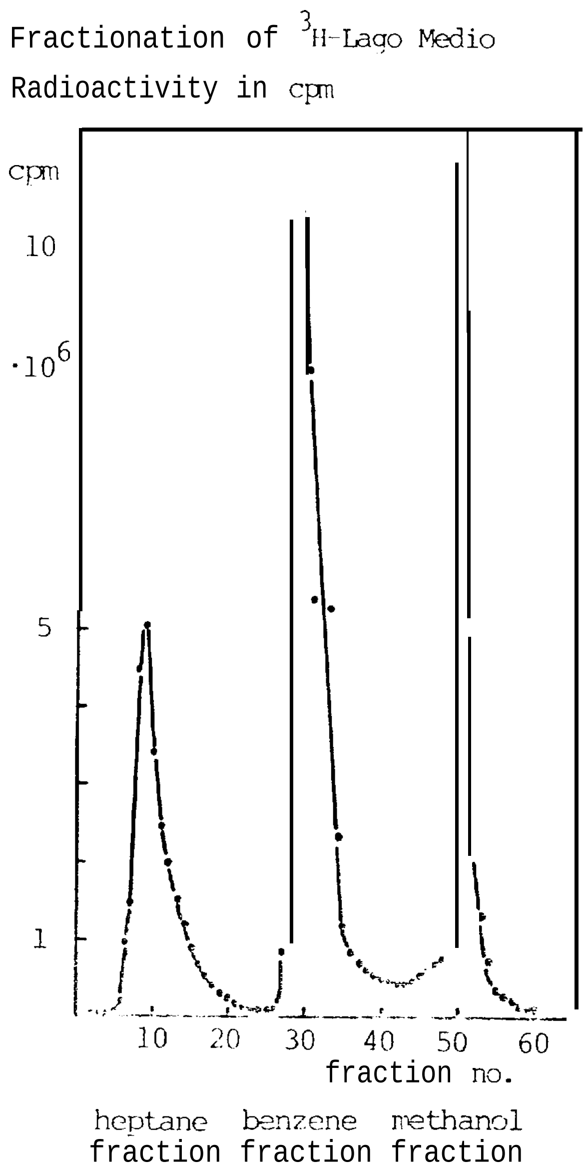


Fig. 2. Chromatographic analyses of generally tritiated Lago Medio crude oil.

Profile of radioactivity in fractions of hydrocarbon eluted from a column of activated silica.

(0.1 to 0.4 ml original sediment volume) were dispensed into 120 mm tubes containing 9 ml of water (10 m, same station).

When the necessary number of identical tubes had been prepared from the same sample, sterile 3 mm cellulose-pads containing known amounts of radioactive oil or hydrocarbon were added, the tubes securely closed and placed radially on a rotating cylinder inside a specially designed water bath. The rotor revolved at approximately 30 rmp.

Due to a failure of the cooling thermostat brought to Cape Hatt a constant temperature of $0^{o}C$ had to be selected as an incubation temperature. Constant temperature was maintained by a mixture of water and crushed ice.

2.2.2.2. Analyses of mineralization products.

At 4-6 intervals over 10-16 days 0.5 or 1.0 ml aliquots of the water phase of each tube were drawn for the extraction and determination of the radioactive mineralization products.

$^{14}CO_2$ were adsorbed by 1 N NaOH applied to a glassfiber filter using standard extracting procedure. After complete absorption the filter was baked at $100^{o}C$ for 6-10 hours to strip off co-adsorbed ^{14}C-hydrocarbons and placed in vials for later counting.

Aliquots from experiments with ^{3}H-Lago Medio were applied to a column of successive layers of Silicone SE-80 (Methyl) and Chromosorb B, BIO-Rad A6 50W x 8 (kation exchange resin), BIO-Rad A6 1 x 8 (anion exchange resin) and activated charcoal mixed with Porapak Type Q (1:1). The column was designed to retain essentially quantitatively all organic radioactive compounds from the tritiated oil or organic products derived from them, leaving $^{3}H_2O$ the only radioactive species of significance. The latter was recovered by elution with 4 one ml portions of distilled water, each collected separately in counting vials; 0.1 ml toluene added and vials kept frozen (when possible) until they were counted.

After return to Trondheim 10 ml Stint Hei 3 was added to each vial and radioactivity of ^{14}C or ^{3}H measured in a Packard model 3375 Liquid Scintillation Spectrometer.

2.2.2.3. Calculation of V_{max}.

For each water or sediment sample to be assessed a set of experiment consisted of 5 tubes identical except for the added hydrocarbon substrate which were present in increasing amounts from tube one to five. The amounts added varied from 5 to 200 μg/tube for substrates with 1% specific activity and from 2 to 50 or 0,5 to 20 μg/tube for substrates with the highest activity.

In successful experiments the accumulation of products directly related to the mineralization of the added hydrocarbon substrate proceeds in a linear fashion, often after a characteristic lag period. In some cases this linear rate is used to express the biochemical activity for hydrocarbon mineralization.

The observed linear rates will increase with increasing amounts of added substrate, when varied within a certain range of concentration. The set of 5 rates for each experiment was used to calculate a maximal rate for mineralization, V_{max}, according to Michaelis-Menten kinetics using the technique described by Wright and Hobbie (1965). This value represents the rate of mineralization at conditions when substrate concentration no longer limits the reaction rate. V_{max} therefore can be considered a term expressing the potential of the microorganisms in the sample for the utilization and mineralization of hydrocarbons and suitable for comparative purposes.

2.2.3. Microbiological analyses.

Water samples were processed immediately after returning to camp. Serial tenfolds dilutions were made in sterile seawater.

Sediment samples from Bay 9 to 11 were used as brought to camp and not homogenized in any way. Subsamples were taken by inserting and filling the barrel of a 5 ml sterile disposable syringe, the tip of which had been cut off to leave full barrel opening. 5 ml sample was mixed with ice-cold sterile 0.1% Tween 80 in seawater and the suspension shaken by hand for 2 minutes. Tenfold serial dilutions of the resulting slurry were made in sterile seawater.

Samples of beach sand and tidalzone sediments were treated with 0.1% Tween 80 in the same manner as for bottom sediments. Sand containing oil needed more than 2 minutes shaking to produce a homogeneous slurry. Seawater - distilled water (1:1) was used for preparing Tween 80 solution and the tenfold dilution series.

2.2.3.1. MPN generally heterotrophic microorganisms (bacteria).

For assessing the total viable count of generally heterotrophic bacteria a liquid most probable number (MPN) technique was used. 3 times 0.1 ml aliquots from each selected dilution were mixed with 0.1 ml medium in 3 wells of presterilized tissue culture plates 3596 (Costar, Cambridge, Mass.). Each plate had 96 wells permitting 4 analyses (each 3x8 microcultures of 0.2 ml). The final growth medium contained per liter: 5 g peptone, 1 g yeast extract, 15 g NaCl, 0.1 g K_2HPO_4, 30 mg phenol red, pH 7.8, in seawater - distilled water (1:1).

Positive cultures were scored by turbidity after incubation for 15 days at 10°C.

2.2.3.2. MPN-oildegrading microorganisms (bacteria).

For assessing a number for oildegrading microorganisms a liquid most probable number (MPN) technique was used, with a mixture of *n*-hexadecane and weathered Lago Medio crude being the selective source for energy and carbon.

Three 1 ml aliquots from each selected dilution were added to three one dram flasks (approx. 5 ml) containing a mineral-hydrocarbon medium which after inoculation had the following composition per 1: 0.1 g NH_4NO_3, 0.1 g K2HP04, 0.05 g phenol red and 25 g of a mixture of weathered Lago Medio crude (weathered to "boiling point" above 200°C) and *n*-hexadecane (1:1) in sea-water, pH adjusted to 7.8-8.0.

10 and 100 ml water samples were filtered through sterile 25 mm polycarbonate membrane filters (Uni-Pore 0.2 µm) and the filters, as inoculum, pushed into the MPN-flasks by a sterile toothpick.

Normally 6 dilutions were used as inoculum for the 3 flask MPN series; for water samples 100, 10, 1, 10^{-1}- 10^{-3} ml served as inoculum and for sediment and sand samples 10^{-1}-10^{-6} dilutions were made with reference to 1 ml of the sample volume. The flasks were incubated at 10°C and scored after 15-20 and 25-30 days. A positive oildegrading culture was assessed by a clear change in the indicator color from red/purple to yellow. Blanks without hydrocarbon substrate will under such circumstances remain unaltered.

This MPN-method for enumeration of oil degrading microorganisms has been used for sample materials giving numbers for oil degrading bacteria ranging from 0.001 to 100% of the viable count for heterotrophic bacteria in the same sample.

The selection of 10°C for incubation temperature is deemed a reasonable compromise between the wish for a procedure with a high degree of analytical expediency and our experience with the requirements of the most sensitive marine psychrophilic microorganisms.

2.2.3.3. MPN-antibiotica resistant oildegrading microorganisms (fungi).

The method was a slight-modification of the described most probable number (MPN) technique for oildegrading microorganisms and aimed at assessing the content of oildegrading fungi in beach sand and tidal zone sediments. The presence of a mixture of three antibiotic were intended to subdue any growth of procaryotic bacteria and thus favor growth of oildegrading fungi. But in addition to creating a more selective medium, a lower analytical sensitivity was expected due to the drastic reduction in the possibilities for secondary growth on intermediate products of the initial hydrocarbon degradation. All cultures that eventually were scored positive with this technique did contain fungi, but bacteria were also almost invariably present. Therefore we prefer the given designation of the method.

Three 1 ml aliquots from each selected dilution were added to three 2 ml tubes containing 0.1 ml medium base and approx. 25 mg of the mixture of weathered Lago Medio crude oil and *n*-hexadecane (1:1). The tubes were closed by sterile plastic caps. After inoculation the medium had the following composition per 1: 0.1 g NH4N03,0.01 g K2HP04, 50 mg each of phenol red, streptomycin, chloramphenicol and tetracycline, in seawater - distilled water (1:1) and pH adjusted to 7.8-8.0.

The MPN-tubes were assessed after 15-20 and 25-30 days at $10^{o}C$.

2.2.4. Measurement of CO_2-production in beach sand and tidal sediment.

The open end of a plexiglass cylinder serving as a C02 measurement cell (see Fig. 3) were pressed firmly into sand or intertidal sediment. The panshaped CO_2-absorption chamber was loaded with 3 ml 1 N CO_2-free NaOH-solution by injection through a diafragm on the top airtight end of the cylinder. A thermometer mounted into the same end permitted the temperature inside the cell to be recorded. To minimize the heating effect of radial energy the cylinders were coated with a reflecting paint.

Over a period of 6-10 (tidal zone) to 20-25 hours (beach sand) samples of the NaOH-solution were drawn by a syringe piercing the needle through the diafragm. Before the actual samples were drawn the content of the absorption chamber was thoroughly mixed by pumping the solution in and out of the syringe. The samples were sealed in glass-ampules until analyzed for C02 in an Envirotech DC50 Total carbon analyzer.

2.2.5. Detritovore invertebrate assessment.

Pit-fall traps containing 3% formaldehyde were mounted in the beach sand with the edge of the trap at level with the surface.

2.2.6. Experimental plots for observation of natural decay of oil.

An area of apparent uniform character in the supralitoral zone of Bay 102 was divided into three plots, each 2x10 m. One area (102B) was sprayed with weathered (~8%) Lago Medio crude oil to give 10 $l \cdot m^{-2}$ and one (102C) with the same oil emulsified with equal volume of water to give 20 $l \cdot m^{-2}$. The technique of spraying is described in the Report of Baffin Island Oil Spill Project - Shoreline component. Interim Report. Woodward-Clyde Consultants. Nov. 28, 1980.

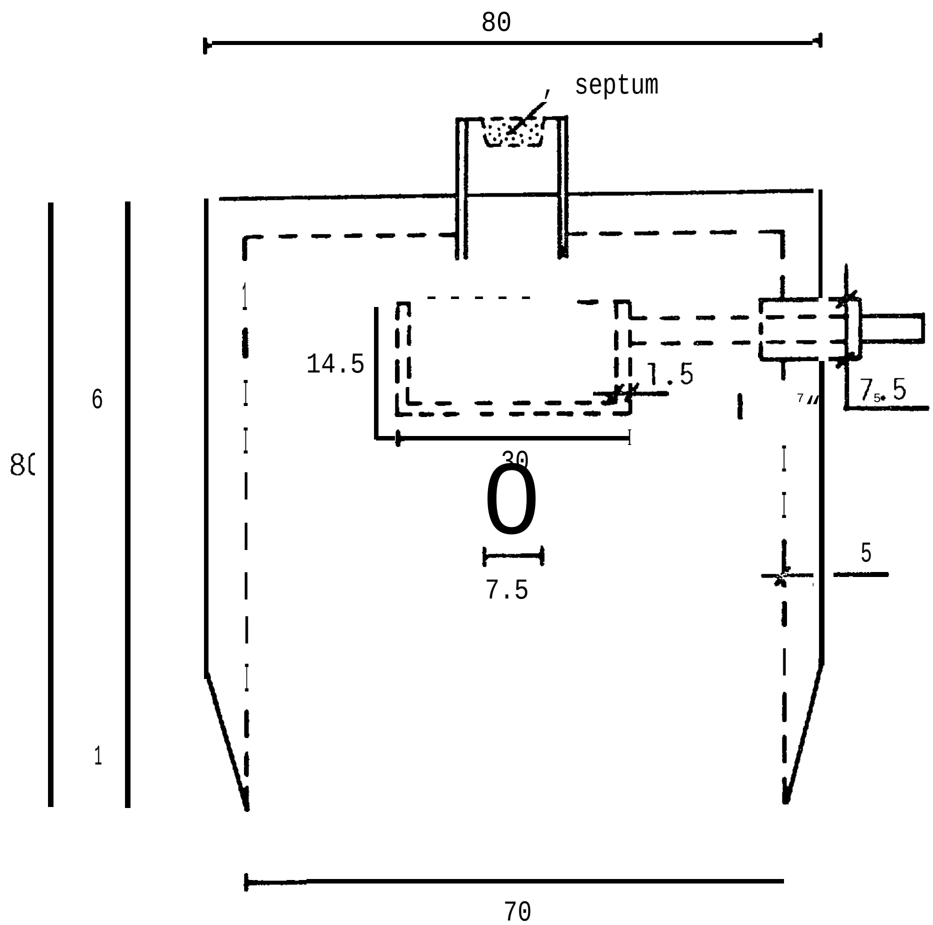


Fig. 3. Absorption chamber for analyses of carbon dioxide in sand and tidal sediments.

All measures in mm.

3, RESULTS AND COMMENTS

Within the framework of the objectives for the BIOS Project the results of our work at Cape Hatt in 1980 constitutes a baseline for the effect studies to follow in 1981 and 82-83. Except for some comments directly relevant to the analytical values obtained, there is therefore no grounds for any extensive discussions in this report.

3.1. NEAR SHORE PROJECT

3.1.1. Analyses of the microbial population in the watercolumn.

Table 1 and Fig. 4 summarize the results of the microbiological analyzes for viable count of generally heterotrophic microorganisms (essentially bacteria) and oildegrading microorganisms (essentially bacteria) in lm, 5m and 10m water samples from the fixed sampling stations in Bay 9, 10 and 11 over the short time period from 28 August to 4 September. The measurements have to be considered an assessment of the level of bacteria present in these waters during the period of highest water temperature (see Appendix with quotations of the results given by Seakem for the environmental physical and chemical parameters) and the fluctuations mainly caused by the short-term physical processes and do not represent any attempt to assess seasonal fluctuations.

A liquid most probable number technique has been used to obtain values for the viable count of generally heterotrophic bacteria. In our hands this technique gives higher values than other techniques used to obtain the same parameter.

The analytical figures for general heterotrophs varied between $1.5\cdot10^5$ and $9.5\cdot10^6$ L^{-1}; in one instance a high $4.5\cdot10^7$ L^{-1} was recorded. The data for total bacteria (direct count using an epifluorescence method) in the same samples were not at our disposal when writing this report, so detailed comparisons cannot be made.

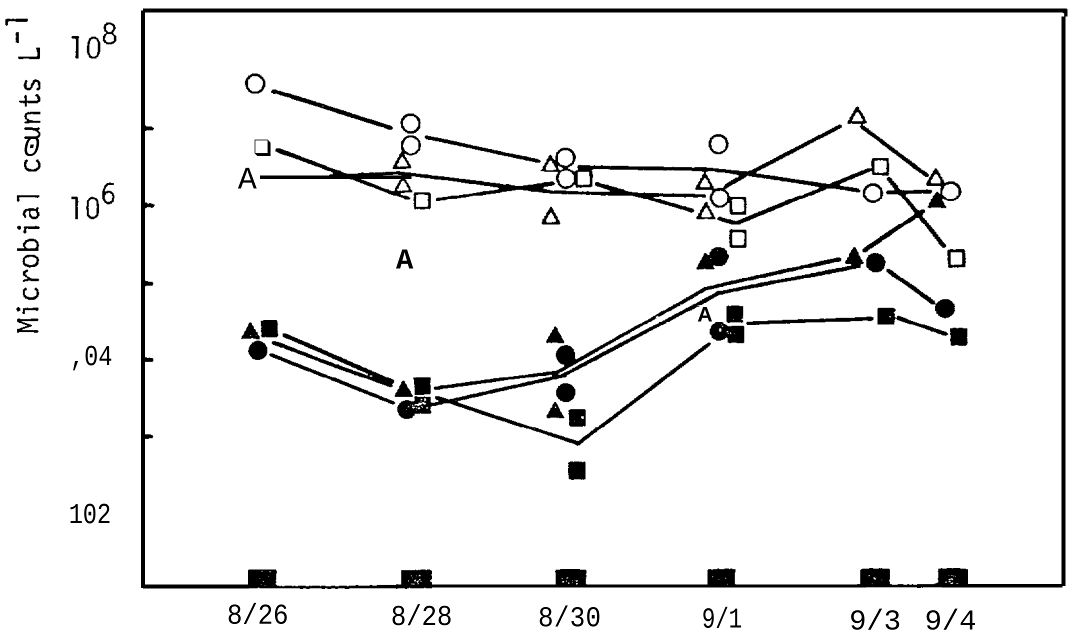
All methods for enumerating socalled oildegrading microorganisms are based on the selectivity of an oil/hydrocarbon substrate and an observable response directly or indirectly connected to the biochemical attack on one or an unknown number of the hydrocarbons in the substrate. The use of MPN methods for this purpose essentially determine the smallest aliquot of the sample that will give a positive response. This minimum requirement for a response is defined as equivalent to one cell and the result of the analysis expressed as a number of oildegrading cells present per unit volume or weight of the sample. The latter definition is most like not true.

For a variety of other reasons the various methods designed will vary considerably in their sensitivity and consequently yield different numerical values when analyzing the same sample. Nevertheless, each method may be fully useful for making conclusions when comparing two or more situations. All methods, however, share the characteristic common to all methods for viable counts of microorganisms, the analytical figures are lower than the actual, true figure.

TABLE 1. Bacterial counts in the nearshore water column of Bays 9, 10 and 11, Ragged Channel, Cape Hatt, Baffin Island; August-September 1980.

Station	(cycle)	Date	Bacteria liter^{-1} Oil degraders	Bacteria liter^{-1} Total heterotrophs
H1	1	8/26/80	$2.5 \cdot 10^{4}$	$2.5 \cdot 10^{6}$
	5	"	9.5″10^{3}	4.5″107
	10	"	2.5⊙10^{4}	7.5″10^{6}
Hi(3)	1	8/28/80	2.5⊙10^{5}	4.5″106
	5	"	2.0″10^{3}	9.5″106
	10	"	4.5″10^{3}	9.5″10^{5}
H2(3)	1	"	4.5″10^{3}	$2.5 \cdot 10^{6}$
	5	"	2.5*10^{3}	7.5″10^{6}
	10	"	2.5⊙10^{3}	1.1″10^{6}
H3(3)	1	8/30/80	2.5⊙10^{3}	7.5″10^{5}
	5	"	4.5″10^{3}	$2.5 \cdot 10^{6}$
	10	"	4.5″10^{2}	$2.5 \cdot 10^{6}$
H4(3)	1	"	$2.5 \cdot 10^{4}$	4.5″106
	5	"	9.5″10^{3}	4.5″106
	10	"	1.5″10^{3}	2.5010′
H5(3)	1	9/1/80	$2.5 \cdot 10^{5}$	2.0″10^{6}
	5	"	$2.5 \cdot 10^{5}$	9.5″105
	10	"	2.5″10^{4}	4.5″10^{5}
H6(3)	1	"	4.5″10^{4}	7.5″10^{5}
	5	"	$2.5 \cdot 10^{4}$	7.5″10^{6}
	10	"	4.5″10^{4}	$9.5 \cdot 10^{5}$
H1	1	9/3/80	2.5″10^{5}	1.5″10^{7}
	5	"	1.5′10^{5}	1.5.10^{6}
	10	"	4.5″10^{L}	4.5″10^{6}
H4	1	9/4/80	1.1″10^{6}	$*2.5 \cdot 10^{6}$
	5	"	4.5″10^{4}	$*1.5 \cdot 10^{6}$
	10	"	$2.5 \cdot 10^{4}$	$*2.5 \cdot 10^{5}$

* Assessed after 10 days of incubation.



Heterotrophs

A 1 m

o 5 m

□ 10 m

Oildegrading

▲ 1 m

● 5 m

■ 10 m

Fig. 4. Analyses of the microbial population in the nearshore waters of Bay 9, 10 and 11 of Ragged Channel, Baffin Island; August/September 1980.

Viable counts for oildegrading and generally heterotrophic bacteria at 1, 5 and 10m.

The number of oildegrading bacteria in the water samples of Bay 9 to 11 found by using our MPN method varied normally between $2.5\cdot10^3$ and 2.5.105 L^{-1}, with an occasional low value of 450 and a high value of 1.1˙106 L^{-1}. This corresponds to concentrations found by us in the Barents Sea using the same method. Bunch (1979) reported the high range values for oleoclastic bacteria in the Davis Strait to the 103 to $10^4 L^{-1}$, with occasional stations with concentrations above 104 L^{-1} (August 1978) In Prudhoe Bay Horowitz and Atlas (1977) recorded high values of 10^3-10^5 oildegrading bacteria per ml during the summer season. The method of analysis were different in all three cases.

For the samples taken during the cycle 3 period an average concentration of $4\cdot10^4$ L^{-1} oildegrading bacteria can be calculated. This means that an average about 1% of the population of the generally heterotrophic bacteria (based on viable count) in the coastal water of Ragged Channel possess the physiological ability to attack hydrocarbons of mineral oil. This does not necessarily implicate that these waters are recipients of mineral oil components. Naturally occurring hydrocarbons different in nature to the hydrocarbons of mineral oil may be responsible.

Most of the highest values for oildegraders (except H1-1m-8/28) occurred during a period of great stratification of the surface water (see figures for salinity in the Appendix). A connection between high surface counts and land drainage cannot be ruled out.

It has to be admitted that the concentration of oildegraders found in the waterphase was somewhat surprising based on otherwise unsubstantiated notions. For this reason some watersamples were taken in the nearshore waters on the west side of the Ragged Channel, in areas assumed to be entirely free of any accidental pollution due to logistic activity. The results of these analyses are given in Table 2. The level of oildegrading bacteria in these samples were the same as found for Bay 9 to 11 and giving no support to any suspicion of contamination of the latter bays.

3.1.2. Analyses of the microbial population of the bottom sediments.

5 sediment samples, at least one from each bay, were analyzed. This was somewhat less than planned. The results are presented in Table 3. Per ml sediment 2.5"10^3 to $2.5\cdot10^4$ oildegrading units could be detected and the viable count of general heterotrophs varied from $2.5\cdot10^5$ to $10^7 ml^{-1}$. Relative to the concentrations in the watercolumn the total bacterial population of the sediments are not high, but there is a considerable variation in the relative proportions between the two groups of bacteria. This needs clarification next year.

3.1.3. Mineralization of t-ago Medio crude oil and defined hydrocarbons.

The method developed to measure quantitatively the biochemical activity for mineralization of oil and hydrocarbons (i.e. oxidation to the products of carbondioxide and water) is particularly designed to assess the low activities associated with the water of open ocean. For this reason we thought the method might be useful for assessment of the oildegradation potential of the cold environment of the Arctic Sea.

TABLE 2. Bacterial counts in nearshore water column at the west side of Ragged Channel (stations S1-S3), Ragged Island, Baffin Island; September 1980.

Station		Date	Bacteria liter^{-1}	
			Oil degraders	Total heterotrophs
S1	1	9/9/80	$2.5 \cdot 10^{5}$	*$1.1 \cdot 10^{6}$
	5	"	$2.5 \cdot 10^{5}$	*$9.5 \cdot 10^{4}$
S2	1		$1.1 \cdot 10^{6}$	*$9.5 \cdot 10^{4}$
	5		$4.5 \cdot 10^{4}$	*$1.5 \cdot 10^{5}$
S3	5	"	$2.5 \cdot 10^{4}$	*$9.0 \cdot 10^{3}$

* Assessed after 5 days of incubation.

TABLE 3. Bacterial counts in nearshore bottom sediments*of Bay 9, 10 and 11 of Cape Hatt, Ragged Channel, Baffin Island; August/September 1980.

Sample H2 collected as indicated, the other samples were taken at 7 m depth close to station indication.

Station	Date	ml "sediment^{-1}	
		Oil degraders	Total heterotrophs
H2(3)	8/29/80	2.5″10^3	$9.5 \cdot 10^6$
H4(3)	8/31 /80	7.5″103	9.5″106
H5(3)	9/2/80	9.5″10^4	2.0″105
H6(3)	·-	9.5″10^3	9.5″10^5
H1 **I	9/2/80	2.5″10^4	*** $2.5 \cdot 10^4$
H1 **II	"	2.5010″	*** $1.5 \cdot 10^5$
H1 **III	"	2,5o10^3	*** $9.5 \cdot 10^4$

* Uncorrected for water content in the sediment.

** I-III successive 1 ml samples drawn from same sediment sample container.

*** Results assessed after only 11 days.

TABLE 4. Mineralization of Venezuelan Lago Medio crude oil at 0°C by microorganisms in water from Bay 9, 10 and 11, Ragged Channel, Cape Hatt, Baffin Island; August/September 1980.

Calculated maximal rates of mineralization (V_{ax}) based on laboratory experiments with radioactive tritiated weathered Lago Medio crude.

Station	Date	V_{max}, µg/m^3,d ^{3}H-Lago	Oil degrading bacteria L^{-1}	Generally heterotrophic bacteria L^{-1}
H2	8/28/80	32	4..5*103	7.5×10^{6}
H4	8/30/80	29	9.5×10^{3}	4.5×10^{6}
H5	9/01 /80	26	2.5×10^{5}	9.5×10^{5}
H1	9/03/80	11	1.5×10^{5}	1.0×10^{6}
H4	9/04/80	13	4.5×10^{4}	$1,0 \times 10^{6}$

The method has certain limitations as to the magnitude of activity of the sample and since the latter was entirely unknown we had to design the experimental program to cover a considerable range to enhance the chance for at least some successful measurements. For this reason concentration of substrate, spesific activity of substrate and to some extent sample size were varied in a systematic manner.

Due to the failure of the low temperature thermostat brought to Cape Hatt to control the temperature of the incubator 0°C had to be selected as temperature of incubation in stead of the *in situ* temperature.

In total 22 experiments were carried out in attempt to assess the mineralization activity of water samples and 13 experiments for assessment of bottom sediments. Generally tritiated Lago Medio crude was used as substrate in 14 experiments and ^{14}C-*n*-hexadecane, naphthalene and benz(a)-pyrene in 21.

Only 5 experiments using ^{3}H-Lago Medio crude oil with the highest specific activity were successful in the sense of giving results permitting calcukaton of V_{max} (rate of mineralization at saturating concentration of oil). The results are given in Table 4.

For convenience the rates of mineralization determined experimentally have been recalculated to µg of oil mineralized per day by the microorganisms in one cubic meter of the sample water. The values for V_{max} determined in this way varied between 11 and 30 µg/m^3, d.

The experimentally determined rates bear little apparent relationship to the content of oildegrading bacteria in the same samples. The recorded V_{max} values corrolate better to the viable count for general heterotroph. Although the degradation of oil obligatory depends on oildegrading bacteria the general heterotrophs may also contribute substantially to the total mineralization.

This apparent lack of correlation between the specific microbiological and biochemical analyses for oil degradation has been observed by Bunch (1979) for a much larger sample material from Davis Strait in 1978 and to a lesser extent for the North Sea. Several possible explanations for this situation can be offered. Bunch favors the idea that the mineralization of *n*-hexadecane in the August water of Davis Strait was primarily limited by nutrients. The water of Ragged Channel was extremely low in nitrate in August-September. For the Barents Sea and the North Sea we nave no experimental grounds for supporting any hypothesis.

Although we have very few measurements of this type to make good comparisons, our measurements at Cape Hatt, Barents Sea and the North Sea have been done with identical methods. Below the results of these analyses are summarized in terms of giving the ranges found for the rate of mineralization of oil (based on the use of tritiated oil) and the content o oildegrading bacteria in the same water sample. The rate of mineralization were determined at somewhat different temperatures in the various investigations and for the Barents Sea samples the rate (V) were calculated from single concentration experiments. The corresponding V_{max} will be somewhat higher.

	Temperature for mineralization rate measurement	Mineralization $\mu g/m^3,d$	Oildegrading bacteria L^{-1}
Barents Sea	$-0.5 - 4.2^oC$	v: 7 - 21.8	$2.5 \cdot 10^3 - 5.5 \cdot 10^5$
North Sea	$8 - 9^oC$	V_{max} : 26 - 114	$9.5 \cdot 10^3 - 4.5 \cdot 10^5$
Ragged Channel	0^oC	v_{max}: 11 - 30	$4.5 \cdot 10^3 - 2.5 \cdot 10^5$

In spite of these differences it can be seen that values for V_{max} found for the Ragged Channel water in a general way fit into expected range based on the content of oildegrading bacteria.

The other experiments with Lago Medio crude oil may also have yielded useful data in spite of a much lower specific activity of the substrate. But contrary to previous experience with titriated Ekofisk crude oil the tritiated Lago Medio preparation gave unduly high background counts and thus lowered the sensitivity of the analysis. We have reason to believe that this problem will be solved prior to the 81 season.

In experiments with the defined ^{14}C-hydrocarbons only the substrates with the highest spesific activity yielded radioactive C02 in measurable amounts, but unfortunately the results could not be used for calculation of V_{max} in the usual manner.

The best indications for mineralization were obtained with ^{14}C-*n*-hexadecane and some rates of mineralization calculated from single concentration experiments are reported in Table 5. Although no strict comparison between V_{max} for one substrate and V for a single concentration for another substrate can be made, the results indicate that the mineralization rate for *n*-hexadecane is slower than for crude oil in water (H4, Table 4). The apparent higher rate observed for the sediment sample is in this case consistent with a higher concentration of oildegrading bacteria in the sediment relative to the water (Table 1 and 3).

in some experiments we have indications for mineralization of naphthalene and benz(a)pyrene, but the results are not consistent and need to be confirmed next year before any conclusions can be made.

TABLE 5. Mineralization of ^{14}C-*n*-hexadecane in water (Bay 10) and sediment (Bay 9) of Ragged Channel, Baffin Island; August/September 1980.

Linear rates of mineralization (V) based on single concentration experiments.

Added *n*-hexadecane µg/tube	Water (H4-8/31) µg/m^3,d	Sediment (H6-9/2) µg/L,d
0.5	0.6	2.5
5	2.6	7.5
10		15.8

3.2. ON SHORE PROJECT

In 1981 a series of experiments will be started to test the effect of fertilizers (nitrogen and phosphate) and mechanical treatment of the sand-oil mixture (increased aeration) on the natural microbial weathering and mineralization of oil stranded on the shoreline. The plots for these experiments were selected this year and baseline analyses of microbial population and biological C02 production were carried out.

It is assumed that the natural processes for oil decay may take a long time in these cold regions and to gain a year of observation, one set of testplots were started in 1980.

3.2.1. Baseline analyses of microorganisms and CO_2-production in shoreline sand and sediments.

The test area in Bay 103 of the Z-lagoon (low energy beach) was monitored for microbial population and CO_2-production over the period from 28 August to 9 September and the results are given in Table 7 and Table 9. The results for the control area (Table 8) may also be included.

The total number of analyses are admittedly low. In the supralitoral area low but detectable numbers of oildegrading bacteria were observed. The count of antibiotic resistant oil degraders (fungi) were even lower. It is, however, expected that this method of analysis may have a low sensitivity, so a direct comparison may not be entirely justified. The analyses for generally heterotrophic bacteria vary considerably, 4.5″103 - $2 \cdot 10^7$ ml. This may reflect the method of sampling. The great abundance of snow-geese droppings in this area may also contribute to the quantitative variability. In the tidal zone (Table 7) the content of oildegrading bacteria were consistently higher and increased quite markedly over the period of sampling. A similar increase in general heterotrophs was not noticeable, but analyses for the last samplingday are lacking. Already before the first sampling sheens of oil were observed in the tidal zone later to be sampled. The oil most likely had drifted to the innermost beaches of 103 from testsites closer to the opening of the bay. These sites were sprayed with Lago Medio crude and emulsified crude during the days of 20-21 August. In spite of the boom some oil may have escaped. Later on (9/20) chemical analyses revealed substantial concentrations of oil in Bay 103 (Seakem, see Appendix). This might offer an explanation for the changes taken place in the microbial flora of the tidal sediments. The rather short responsetime is interesting.

As an overall parameter for the biological activity of the shoreline sand and sediments we have attempted to measure the total CO_2 produced from the biota restricted to a certain volume of sand in communication with a bell-shaped absorption chamber (Fig. 3). Table 9 reports characteristic values obtained for CO_2-production rates in selected areas of apparently uncontaminated beach sand and tidal sediments. A clearcut difference was found between activity in the sand of high and low energy beaches. Analyses of chemical and physical parameter in the sand (Table 6) offer no explanation for the observed differences in biological activity.

During the last measurements on the high energy beach (Bay 102) the temperature in the top layer of the sand dropped to -0.4oC. This temperature decrease did not seem to reduce the rate of C02 production noticeably (see Bay 102 D-H, Table 9).

3.2.2. Natural decay of oil on beach sand - Initial observations.

In the supralitoral zone of Bay 102 plots (2x1O m) were sprayed to give a 1 cm layer of weathered Lago Medio crude or a 2 cm layer of 50% emulsified Lago Medio crude oil. A nearby plot was selected as a control. The plots were oiled 24 August and left undisturbed for observation of the natural decay of oil and for comparison of the natural decay of emu" sified and not emulsified oil. At the same time these plots will serve as respective controls for series of experiments on enhancement of natura decay of oil to be started in 1981. The former experiments were started this year to leave *us* the longest possible period of observation.

The results of the microbiological analyses for the initial 2 weeks after oildeposition are given in Table 8. In the same period the rate of CO_2-production was measured 3 times and the results are presented in Table 10. Analyses of the sand in plot B and C prior to the deposition of the oil are lacking. Two days after the deposition of the oil the levels of general heterotrophic and oildegrading bacteria were still rather low and comparable to the level in the control plot. The oil itself thus did not seem to contribute significantly to the microbial population.

7 and 15 days after the spill decisive increases in the population of oil-degrading as well as generally heterotrophic bacteria seemed to have taken place. There was a particularly pronounced development of oildegraders in the sand containing the emulsified oil and the same may have been the case for the general heterotrophs. For logistic reasons the incubation period for the analyses of the latter was too short and the counts may certainly be on the low side. The counts for antibiotic resistant oildegrading microorganisms remained low, with the exception of a possible small increase in the emulsified oil plot.

Admittedly the number of analyses are few and the observed difference in the microbial development in the two types of oil needs confirmation. The weather during the period of observation was extremely good with hardly any percipitation, and maximal temperatures of 17-18^{o}C were measured in the top surface in both oiled plots. Under these conditions it is reasonable to assume that the high watercontent -of the oil emulsion may have created a more congenial environment for bacterial growth. The effect of water activity in the oil on the microbial growth will be looked into more carefully next year.

The observed rates of CO_2-production dropped markedly when the sand was covered by oil (Table 10). This is commonly observed when massive amounts of oil penetrated down into sand or soil and it is generally assumed to be caused by the toxic effect of oil on the biota. The plot oiled by weathered Lago Medio crude appeared to recover after a few days, but the CO_2-production in the plot with emulsified oil remained low during the whole period. This was in apparent contrast to the microbial development in the same plots.

A direct comparison of the plots entirely based on the observed rate of C02 production may not be warranted. The field method used to determine C02 is obviously dependent on the total volume of sand/sediment with biota that contribute CO_2 to the absorption chamber. A massive addition of oil to the sand will reduce this volume more or less as well as affecting its biota. A visual inspection of the oiled plots clearly indicated a pronouned difference between the two plots in this respect. The sand sprayed with weathered Lago Medio crude oil appeared to retain its characteristic airy and rather loose structure whereas the emulsified oil to a great extent remained on the top of the sand forming a continuous liquid surface. The latter most likely formed a boundary that will reduce the volume in diffusion contact with the CO_2-trap. The observed rates of CO_2-production in the two oiled plots are therefore not necessarily comparable, and not representative for, the respective microbial activity.

3.2.3. Conclusions.

The beach sand contained low, but detectable levels of oildegrading microorganisms. A week after deposition of oil an increased level of these bacteria were noticeable, in spite of low levels of nitrogen and phosphor. Under the dry conditions of the arctic summer the emulsified oil seemed to offer the best substratum for bacterial development.

TABLE 6. Chemical and physical properties of beach sand, Cape Hatt, Baffin Island; August/September 1980.

Bay	Water %	Organic matter mg/g	Total nitrogen mg/g	Total phosphor mg/g	Nitrate µg/g	Phosphate µg/g	pH
102	0.8	4	1.1	0.13	1	<0.5	8.9
103	17	8	0.8	0.14	0.6	<0.5	7.9

TABLE 7. Baseline levels of microbial activity in sand and sediment of the shoreline of Z-lagoon, Cape Hatt, Baffin Island (Bay 103, low energy beach); August/September 1980.

Counts of generally heterotrophic bacteria, oildegrading bacteria and antibiotic resistant oildegrading microorganisms.

	Cells per ml sample					
	Supralitoral zone		Midtidal zone		Low tidal zone	
Sampling date	Oil degraders	Hetero-trophs	Oil degraders	Hetero-trophs	Oil degraders	Hetero-trophs
8/29/80	95(25*)	2“10^{7}	450(25)	2.5o10^{5}	450 (25)	9.5910^{5}
9/02/80	950(25)	4.5”10^{4}	3.5”10^{3}(25)	9.5”10^{4}	2o10^{3}(250)	2*10^{4}
9/09/80	300(25)	-	1.101O’(25)	-	9.5o10^{4}(25)	

* Number in parethesis - antibiotic resistant oildegrading microorganisms.

Numbers not corrected for water content.

TABLE 8. Microbial activity in beach sand after spraying with oil. Bay 102, Cape Hatt, Baffin Island; August/September 1980.

Plot B sprayed with 1 cm layer of weathered Venezuelan Lago Medio crude and plot C with 2 cm layer of 50% emulsion of the same oil; spraying date 8/24/80.

	Cells per ml sand					
	Control Area		Plot B		Plot C	
Date	Oil degraders	Hetero-trophs	Oil degraders	Hetero-trophs	Oil degraders	Hetero-trophs
8/26	0(0)	4..5″103	2*102(O)	7.5″10^{3}	1.5″10^{2}(0)	1.5″104
8/31	95(25*)	1.5″104	2.5o10^{4}(25)	2.5″10^{5}	7.5″10^{5}(250)	9.5″10^{6}
9/08	9(25)	**.2.5″10^{2}	9.5•10^{4}(25)	**4.5•10^{5}	1.5″10^{7}(400)	**4.5″10^{7}

* Numbers in parenthesis - antibiotic resistant oildegrading microorganisms.

** Assessed after only 6 days of incubation at 10^{o}C.

TABLE 9. CO_2-production in beach sand and in tidal sediments at Cape Hatt, Baffin Island; August/September 1980.

The average rate of CO_2-production measured over minimum periods of 24 hours (supralitoral areas) or 6-10 hours (tidal areas).

Bay	Temperature (average mean) °C	$mgC \cdot m^{-2} \cdot h^{-1}$*	Beach type
103A	8.8	11 ± 4.6(4)	supralitoral
103B	11.4	6 ± 3.6(3)	midtidal
103C	10.6	8 ± 2.2(3)	lowtidal
103A	4.8	9.8 ± 3.7(5)	supralitoral
102A	5.8	28 ± 6 (4)	supralitoral
102D-H	0.4	47 ± 19(10)	supralitoral

* Number in parenthesis gives number of measurements.

TABLE 10. CO_2-production in oiled beach sand of Bay 102, Cape Hatt, Baffin Island; August/September 1980.

Plots oiled 8/24 with 1 cm layer of weathered Venezuelan Lago Medio crude (Plot B) and 2 cm layer of 50% emulsion (Plot C) of the same oil.

Days after oiling	Temperature max. variation (mean temp.)	$mgC \cdot m^{-2} \cdot h^{-1}$ *		
		Control plot	Plot B	Plot c
2	3.5-7 (5.6-6.3)	28 ± 6(4)	17 ± 4(3)	13 ± 5(3)
7	3.5-18.5 (10-10.6)	34 ± 2(2)	35 ± 6.5(4)	13 ± 13(4)
12	3.0-16 (7.9-8.9)	12 ± 2(2)	21 ± 8(4)	18 ± 12(4)

* Number in parenthesis indicates parallel measurements.

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APPENDIX

Environmental chemistry and hydrocarbon analyses of direct interest to the present report.

Data from:

Baffin Island Oil Spill Project

Chemistry Component: Baseline year

Seakem Oceanography Ltd., 12 January 1981.

ENVIRONMENTAL CHEMISTRY DATA FOR STATIONS OF BAY 9, 10 AND 11 DURING THE PERIOD OF CYCLE 3 AND 4.

Station	depth	Date	Temp. °C	Salinity 0/00	Nitrate µg at. l^{-1}	Phosphate µg at. l^{-1}	DOC mg l^{-1}	*Particulate org. carbon, µg l^{-1}
	1		3.8	25.5	0	0.53	3.03	140
H1	5	8/28	3.3	26.3	0	0.59	2.13	110
	10		2.6	29.4	0.5	0.81	2.11	100
	1		3,8	24.7	0.1	0.54	2.61	130
H2	5	"	3.0	27.4	0.1	0.48	2.63	180
	10		2.5	29.6			4.09	120
	1		4.1	20.4	0.1	0.38	2.39	190
H3	5	8/30	3.8	24.2	0		2.98	210
	10		3.4	26.7	0.2	0.58	2.72	170
	1		4.2	20.3	0	0.31	~5	140
H4	5	"	3.9	23.7	0	0.44	2.75	140
	10		3.4	26.7	0.1	0.48	2.25	120
	1		4.6	22.8	0	0.37	2.37	160
H5	5	9/01	4.2	23.1	0	0.49	2.42	240
	10		3.3	27.0	0	0.58	2.11	160
	1		4.6	19.6	0	0.56	2.97	140
H6	5	"	4.0	24.0	0.2	0.50	3.56	190
	10		3.0	27.4	0	0.32	2.21	200
	1		4.5	20.2	0	0.36	2.08	150
H1	5	9/05	3.2	27.1	0	0.62	2.50	170
	10		2.0	30.0	0.3	0.76	1.97	170
	1		4.5	18.8	0.1	0.33	2.07	120
H4	5	9/07	3.3	27.2	0.2	0.60	3.03	140
	10		2.0	29.2	0.1	0.71	2.84	200

* Analyzed by Arctic Biological Station, DFO

HYDROCARBONS IN THE WATER OF BAYS OF CAPE HATT, 1980. (Seakem, 1981 P. 66-67)

Date	Bay	Depth	Total hydrocarbons µg L^{-1} *	Comments
8/26	9	1 m	D.L.**	August sampling
		5 m	"	
		10 m	"	
"	10	1 m	26	
		5 m	D.L.	
		10 m	"	
"	11	1 m	D.L.	
		5 m	"	
9/20	9	1 m	D.L.	September sampling
		5 m	"	
9/19	10	1 m	D.L.	
		5 m	"	
		10 m	80	
9/18	11	1 m	D.L.	
		5 m	72	
		10 m	1138	
8/18	103	1 m	D.L.	Prespill
		7 m	"	
8/21	103	1 m	D.L.	Prespill
		7 m	"	
9/20	103	1 m	150	Postspill
		5 m	D.L.	

* µg L^{-1} Lago Medio crude equivalents.

** D.L. = below detection limit of 13 µg L^{-1}.

ANALYSIS OF SEDIMENTS OF BAY 9, 10 AND 11.

Analysis of nutrients
(calculated from data p.61 , Seakem 1981)

Station	Date	Total organic carbon % d.w.	Interstitial nitrate μg at. l^{-1}	Interstitial phosphate μg at. l^{-1}
H2	8/29	N.A.	N.A.	N.A.
H4	8/31	N.A.	3.1	12.0
H1	9/02	0.42	N.A.	N.A.
H5	"	0.30	2.5	9.0
H6	"	0.35	1.1	20.1

Hydrocarbons
(calculated from data p.68-69, Seakem 1981)

Date	Bay	Total hydrocarbons $\mu g\ g^{-1}$ min - max	Total hydrocarbons $\mu g\ g^{-1}$ average	Non-polar hydrocarbons $\mu g\ g^{-1}$ min - max	Non-polar hydrocarbons $\mu g\ g^{-1}$ average
9/12/80	9	7.1-35.9	20.1±10.4	0-2.0	0.8±0.8
9/13/80	10	7.2-24,5	12.7± 6.6	0-2.6	0.8±0.9
9/11/80	11	10.9-26.7	16.5± 6.4	0-1.5	0.9±0.7